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| EXAMINER | |
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| WOOLWINE, SAMUEL C | |
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| SHORTENED STATUTORY PERIOD OF RESPONSE | MAIL DATE | DELIVERY MODE |
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**Please find below and/or attached an Office communication concerning this application or proceeding.**

If NO period for reply is specified above, the maximum statutory period will apply and will expire 6 MONTHS from the mailing date of this communication.

| Office Action Summary | Application No. | Applicant(s) | |
|---|---|---|---|
| | 10/783,884 | SCOTT ET AL. | |
| | Examiner | Art Unit | |
| | Samuel Woolwine | 1637 | |

*-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --*

**Period for Reply**

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

**Status**

1)☒ Responsive to communication(s) filed on *27 October 2006*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

**Disposition of Claims**

4)☒ Claim(s) *25-44 and 55-102* is/are pending in the application.

4a) Of the above claim(s) *69,70 and 89* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *25-44,55-68,71-88 and 90-102* is/are rejected.

7)☒ Claim(s) *72* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

**Application Papers**

9)☒ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

**Priority under 35 U.S.C. § 119**

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

**Attachment(s)**

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *See Continuation Sheet*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

Continuation of Attachment(s) 3). Information Disclosure Statement(s) (PTO/SB/08), Paper No(s)/Mail Date :10/12/04,5/20/05,2/3/06,8/16/06.

## DETAILED ACTION

### *Election/Restrictions*

Applicant's election of Group II, claims 25-44 and 55-99 in the reply filed on 10/27/2006 is acknowledged. Because applicant did not distinctly and specifically point out the supposed errors in the restriction requirement, the election has been treated as an election without traverse (MPEP § 818.03(a)). Applicant's species election of SEQ ID NOS: 1, 14, 15 and 16 is acknowledged. Therefore, unless a generic claim is found allowable, examination of claims 59, 60 and 77-79 will be limited to the elected SEQ ID NOS only. Furthermore, claims 67-74 and 80-94 encompass patentably distinct species (i.e. genes or gene sets). Based on Applicant's election of SEQ ID NO:1 (corresponding to intron 1 of the CEGP1 gene, see figure 1A), examination of claims 67-74 and 80-94 will be limited to a gene or gene set comprising CEGP1 if no generic claim is found allowable. Claims 69, 70 and 89 are withdrawn as non-elected inventions, since these claims do not list CEGP1. This requirement for restriction and election of species is made FINAL.

### *Status*

Claims 1-24 and 45-54 have been cancelled by Applicant's amendment. Claims 25, 39-43, 55, 63, 73, 75, 77-80, 87, 90, 91, 97-99 have been amended. New claims 100-102 have been added. Currently, claims 25-44 and 55-102 are pending and under consideration. Of these, claims 69, 70 and 89 are withdrawn, being directed to a non-elected invention.

### *Claim Objections*

Claim 72 is objected to under 37 CFR 1.75(c), as being of improper dependent form for failing to further limit the subject matter of a previous claim. Applicant is required to cancel the claim(s), or amend the claim(s) to place the claim(s) in proper dependent form, or rewrite the claim(s) in independent form. The limitations of claim 72 are already found in claim 71.

### *Specification*

The disclosure is objected to because of the following minor informality. The second sentence of paragraph [0109] as originally filed begins: "Three 10 μM sections were cut..." However, μM is a unit of concentration, which does not make sense in this context. This will be considered a minor typographical error, and a correction to μm would not be considered new matter. Appropriate correction is required. See MPEP § 608.01(b).

### *Claim Rejections - 35 USC § 112*

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claim 33 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. It cannot be determined from the claim whether "is fragmented" requires an active method step of fragmenting the RNA, or whether the method of claim 32 is simply applied to a sample containing fragmented RNA.

Claim 55 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. It cannot be determined from the claim whether "the expression of which" refers to the intronic RNA, or to the RNA comprising intronic RNA. Either interpretation will be considered for purposes of further examination.

Claims 75-88 and 90-100 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 75-88 and 90-100 provide for the use of a plurality of polynucleotides for measuring gene expression, but, since the claim does not set forth any steps involved in the method/process, it is unclear what method/process applicant is intending to encompass. A claim is indefinite where it merely recites a use without any active, positive steps delimiting how this use is actually practiced. Furthermore, while the specification provides exemplary uses of polynucleotides, there is no explicit definition of "using".

Claims 75-88 and 90-100 are rejected under 35 U.S.C. 101 because the claimed recitation of a use, without setting forth any steps involved in the process, results in an improper definition of a process, i.e., results in a claim which is not a proper process claim under 35 U.S.C. 101. See for example *Ex parte Dunki*, 153 USPQ 678 (Bd.App. 1967) and *Clinical Products, Ltd.* v. *Brenner*, 255 F. Supp. 131, 149 USPQ 475 (D.D.C. 1966).

Claims 77-79 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. It cannot be understood what is meant by the phrase "The method of claim 75 comprising...amplicons...". How does a method comprise amplicons? Does this mean claims 77-79 comprise the "use" of amplicons, which in itself is vague and indefinite? Are the amplicons used as targets? Probes? These claims will be broadly interpreted for purposes of further examination.

Claims 97-100 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claim 100 recites "the plurality of polypeptides" in reference to claim 75. Claim 75 does not recite a plurality of polypeptides. For purposes of further examination, "the plurality of polypeptides" of claim 100 will be interpreted to mean "the plurality of polynucleotides".

Claims 98 and 99 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The claims recite "a 100 μ section". However, μ has no units or dimensions. The specification was searched for possible explanation, but the same use of μ was found in paragraph [0036] and claims 98 and 99 as originally

filed. Therefore, it is unclear what μ means. It could be μm, $\mu m^2$, $\mu m^3$, or even μl or μg. For purposes of further examination, μ will be broadly interpreted.

***Claim Rejections - 35 USC § 112***

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claim 97-100 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. Newly added claim 100 recites "the plurality of polypeptides is immobilized on a solid surface in an array". The original disclosure does not recite polypeptides immobilized on a solid surface in an array. This is a NEW MATTER rejection.

***Claim Rejections - 35 USC § 102***

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 75-88 and 90-100 are rejected under 35 U.S.C. 102(b) as being anticipated by Fodor et al (US 2001/0053519 A1).

With regard to claim 75, Fodor teaches *a method for measuring gene expression* (e.g., first sentence of paragraph [0003]) *using a plurality of polynucleotides* (see paragraph [0003]) *capable of hybridizing to target genes of interest* (any polynucleotide is "capable of hybridizing" to its complementary sequence), *wherein at least one of the said polynucleotides comprises an intron-based sequence the expression of which correlates with the expression of a corresponding exon sequence*. With regard to the *wherein* limitation, Fodor teaches an array of every possible 10-mer oligonucleotide (see figures 2-5 and Example 2, paragraphs [0122]-[0124]). Since every possible sequence of 10 nucleotides is represented on the array, the array would inherently comprise *an intron-based sequence the expression of which correlates with the expression of a corresponding gene.*

With regard to claim 76, an intron sequence can be a dinucleotide, since the claim does not specify that all of said polynucleotides comprise *complete* or *full* intron sequences. There are $2^4$ possible dinucleotides: AA, AC, AG, AT, CA, CC, CG, CT, GA, GC, GG, GT, TA, TC, TG, and TT. The intron shown in Applicant's SEQ ID NO:1 contains each of these dinucleotides. Therefore, since each of Fodor's 10-mers must contain a dinucleotide, and all dinucleotides are "intron sequences", Fodor's complete 10-mer array meets the limitations of the claim.

With regard to claims 77-79, Fodor's complete 10-mer array inherently comprises probes for each of the amplicons listed in figures 1A-M.

With regard to claims 80-88 and 90-92, Fodor's complete 10-mer array inherently comprises probes hybridizing to all of the genes listed in claims 80, 84, 88 and 90-92.

There is no explicit definition of the term "intron-based" in the disclosure of the instant application, and as explained above, not only would each 10-mer found in any intron be represented on Fodor's array, but each 10-mer on the array would also comprise "intron sequences".

With regard to claims 93 and 94, Fodor's complete 10-mer array inherently comprises probes for each of the genes listed in figure 6.

With regard to claims 95 and 96, for any possible gene having introns and exons, Fodor's complete 10-mer array would have a probe complementary to an intron as well as a probe complementary to an exon from that gene.

With regard to claims 97-99, Fodor's complete 10-mer array would comprise probes for every possible gene. Applicant's claims 97-99 are herein relied upon as evidence that at least 150 genes exist. Regarding the limitations reciting some number of genes in a "100 μ section", Fodor teaches that current photolithography can attain resolution of 1 μm × 1 μm (see last sentence of paragraph [0123]), which would allow for 100 × 100 = 10,000 gene (i.e. gene probes) per 100 μ[$m^2$] section.

With regard to claim 100, Fodor's complete 10-mer array contains polynucleotides immobilized on a solid surface in an array (see figures 2-5 and Example 2, paragraphs [0122]-[0124]).

Claims 25, 27-30, 34-36 and 101 are rejected under 35 U.S.C. 102(b) as being anticipated by Coleclough et al (1984).

With regard to claim 25, Coleclough teaches *a method for measuring gene expression* (Northern blot, see figures 1-4) *in a biological sample* (murine B cells and plasmacytoma, see first section of "Results" page 2018), *comprising:*

*(a) providing a polynucleotide complementary to an intronic RNA sequence within a target gene* (see for example figure 1B and caption: "Northern blots of poly(A)$^+$ and poly(A)$^-$ nuclear RNA probed with the intronic *Bst*EII fragment."),

*wherein the expression of said intronic RNA sequence correlates with the expression of an exonic mRNA sequence within said gene* (This limitation is both inherent and taught by Coleclough. It is inherent because the steady-state level of any intron would correlate with the steady state level of any exon from the same gene, as the two are transcribed as a unit and thus have the same rate of synthesis. The steady state level of a sequence, whether intron or exon, would be determined by its rate of synthesis and its rate of degradation. Therefore, even if the intron sequence degraded more rapidly than the exon sequence, the steady state levels of intron and exon would still correlate. For example, a given intron/exon pair might have a correlation of 1:5; i.e. the steady state level of the exon is 5 times greater than the steady state level of the intron. Evidence of this relationship is provided by Coleclough, who states in the abstract that "[a] high level of κ gene expression is not a prerequisite of a cell containing detectable free κ introns; the lymphoma 38c has only 5% or less of the amount of κ mRNA that the plasmacytoma MCP-11 contains, yet the ratio of free intron to mRNA precursor is about the same in both cell lines" (emphasis provided));

*(b) hybridizing said polynucleotide to said intronic RNA sequence to form a polynucleotide-intronic RNA complex* (see figures 1-4 and page 2018, column 1, "Electrophoresis and hybridization");

*(c) detecting the polynucleotide-intronic RNA complex* (see figures 1-4).

With regard to claim 27, as seen in figure 1, for example, the intron sequences under investigation are well above 50 nucleotides long.

With regard to claim 28, the term "tissue" is not defined in the specification. The lymphoid cells used by Coleclough qualify as "tissue".

With regard to claim 29, according to paragraph [0143] of the published instant application: "The term "tumor," as used herein, refers to all neoplastic cell growth and proliferation, whether malignant or benign, and all pre-cancerous and cancerous cells and tissues." Thus, plasmacytoma and B lymphoma cells (see page 2017, column 2, "Cells") qualify as tumors.

With regard to claim 30, according to paragraph [0144] of the published instant application: "The terms "cancer" and "cancerous" refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth." Thus, plasmacytoma and B lymphoma cells (see page 2017, column 2, "Cells") qualify as cancer.

With regard to claim 34, Coleclough teaches cell suspensions, which is a fluid (see page 2017, column 2, first sentence of "Cell fractionation and RNA isolation").

With regard to claim 35, the hybridization taught by Coleclough are stringent conditions (see page 2018, column 1, last paragraph of "Electrophoresis and hybridization").

With regard to claim 36, Coleclough compares the levels of κ gene transcripts in two cell types: "the lymphoma 38c has only 5% or less of the amount of κ mRNA that the plasmacytoma MCP-11 contains, yet the ratio of free intron to mRNA precursor is about the same in both cell lines" (see abstract, see also page 2021, last sentence prior to "Discussion"). This can be regarded as a quantitation.

With regard to claim 101, Coleclough "uses" both intron-based and exon-based polynucleotide sequences (see page 2019, column 1, second paragraph under "Free introns hybridize only with intronic probes and may exist in two conformations", and see figure 2 and caption).

***Claim Rejections - 35 USC § 103***

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under

37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

The factual inquiries set forth in Graham v. John Deere Co., 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

Claims 25, 27-30 and 33-44 are rejected under 35 U.S.C. 103(a) as being unpatentable over Fodor et al (US 2001/0053519 A1) in view of Duvick (USPN 7,026,123 filed August 28, 2002, priority date August 29, 2001).

With regard to claim 25, Fodor teaches *a method for measuring gene expression in a biological sample* (see paragraph [0003]), *comprising:*

*(a) providing a polynucleotide complementary to a sequence within a target gene, wherein the expression of said sequence correlates with the expression of an mRNA sequence within said gene* (see paragraph [0081]);

*(b) hybridizing said polynucleotide to said sequence to form a polynucleotide-sequence complex* (see paragraph [0081]); *and*

*(c) detecting the polynucleotide-sequence complex* (see paragraph [0081]). Note also that Fodor teaches a complete 10-mer array. This array would inherently provide polynucleotides complementary to all possible introns.

With regard to claim 27, Fodor teaches an array of every possible 10-mer oligonucleotide (see figures 2-5 and Example 2, paragraphs [0122]-[0124]). Since every possible sequence of 10 nucleotides is represented on the array, the array would inherently comprise probes for any possible intron sequence, including intron sequences at least 50 nucleotides long.

With regard to claim 28, Fodor teaches tissue (see paragraph [0055]).

With regard to claims 29 and 30, Fodor teaches analyzing hybridization patterns of sample with cancer (see end of paragraph [0087]; cancer qualifies as a tumor tissue).

With regard to claim 33, Fodor teaches fragmenting RNA (see paragraph [0118]).

With regard to claim 34, Fodor teaches biological fluids (see paragraph [0055]).

With regard to claim 35, Fodor teaches stringent hybridization conditions (see paragraphs [0031] and [0120] for example).

With regard to claim 36, Fodor teaches quantifying (see paragraph [0121 for example]).

With regard to claim 37, Fodor teaches single-stranded oligonucleotides (see for example paragraphs [0023], [0025], [0028], [0030] and [0033]).

With regard to claim 38, Fodor teaches the nucleic acid sequences of his invention may be used as probes, primers for PCR, or ligands (see paragraph [0003]).

With regard to claims 39-42, Fodor teaches simultaneous screening of up to 100,000 different hybridizations (see paragraph [0037]). Additionally, Fodor's complete 10-mer array would comprise probes for up to $4^{10}$ (or 1,048,576) genes (see figures 2-5 and Example 2, paragraphs [0122]-[0124]).

With regard to claim 43, Fodor teaches probes immobilized on a solid surface (see figures 2-5 and Example 2, paragraphs [0122]-[0124]).

With regard to claim 44, Fodor's complete 10-mer array would necessarily comprise probes to every gene listed in figure 6, including probes to intron sequences from these genes.

Fodor does not teach or suggest measuring gene expression by specifically using polynucleotides complementary to intron sequences (even though Fodor's complete 10-mer array as well as other n-mer arrays taught by Fodor in paragraphs [0101]-[0103] would inherently comprise polynucleotides complementary to all possible intron sequences).

Duvick teaches detecting intron RNA and that the level of detected intron RNA would be proportional to (i.e. correlate with) the transcription rate (see column 5, last paragraph, continuing in column 6).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention of the instant application was made to incorporate the suggestion of Duvick (to detect gene expression by detecting intron sequences) into the general method taught by Fodor for using oligonucleotide arrays to measure gene expression. Duvick teaches that "[r]ecent data from mammalian cells indicates that intron RNAs,

after splicing, persist in the cell with reasonable half-lives, contrary to what was previously thought" (column 5 line 67 through column 6 line 3). Therefore, the idea of measuring gene expression by detecting intron RNA was clearly known in the art. One would have been motivated to choose intron RNA when practicing the general method taught by Fodor, because in light of Duvick's disclosure, intron RNA sequences simply represent equivalents of the exon RNA sequences for the purpose of detecting an RNA transcript.

Furthermore, Fodor defines mRNA for purposes of his disclosure in paragraph [0038] as follows:

"The term "mRNA" refers to transcripts of a gene. Transcripts are RNA including, for example, mature messenger RNA ready for translation, products of various stages of transcript processing. Transcript processing may include splicing, editing and degradation."

Thus Fodor's view of mRNA includes "products of various stages of transcript processing", and one of ordinary skill in the art would have known that such products include intronic sequences.

Claims 25, 28-30, 32, 33, 36-39, 44, 55-58, 61-66 and 102 are rejected under 35 U.S.C. 103(a) as being unpatentable over Dannenberg et al (US 2001/0029018 A1) in view of Duvick (USPN 7,026,123 filed August 28, 2002, priority date August 29, 2001). Dannenberg discloses a method for extracting total RNA from formalin-fixed, paraffin-

embedded tumor biopsy tissue, followed by reverse transcription and real-time PCR to quantitate gene expression of specific genes.

With regard to claim 25, Dannenberg teaches *a method for measuring gene expression in a biological sample* (see paragraph [0021], for example), *comprising:*

*(a) providing a polynucleotide complementary to a sequence within a target gene, wherein the expression of said sequence correlates with the expression of an mRNA sequence within said gene* (see paragraph [0021] and paragraphs [0049]-[0058]; polynucleotides complementary to a target gene are listed in paragraph [0055]);

*(b) hybridizing said polynucleotide to said sequence to form a polynucleotide-sequence complex* (see paragraph [0054]; primers and probes in real-time PCR hybridize to their targets); *and*

*(c) detecting the polynucleotide-sequence complex* (see paragraph [0054]).

With regard to claims 28-30, Dannenberg teaches an example involving colon cancer tumor tissue (see Example 6, paragraphs [0088]-[0089] and figure 8).

With regard to claim 32, Dannenberg teaches extracting total RNA from formalin-fixed, paraffin-embedded tumor biopsy tissue (see for example paragraph [0021]).

With regard to claim 33, Dannenberg teaches extracting total RNA from formalin-fixed, paraffin-embedded tissue (see paragraphs [0021], [0051] and [0053]). Such RNA tends to be highly fragmented (see paragraph [0003] of instant specification). Therefore, this limitation is considered inherent to the disclosure of Dannenberg.

With regard to claim 36, Dannenberg teaches quantitating gene expression (see Example 6, paragraphs [0088]-[0089] and figure 8).

With regard to claims 37 and 38, the PCR primers and probes listed by Dannenberg are single-stranded.

With regard to claim 39, Dannenberg measures the expression of more than one target gene (see Example 6, paragraphs [0088]-[0089] and figure 8).

With regard to claim 44, Dannenberg measures expression of TS, TP, COX2 and VEGF, all of which are listed in figure 6 of the instant application (see Example 6, paragraphs [0088]-[0089] and figure 8).

With regard to claim 55, Dannenberg teaches *a method for amplifying RNA in a fixed paraffin-embedded tissue sample representing at least one gene of interest, comprising the steps of:*

*(a) contacting DNA obtained by reverse transcription of RNA comprising intronic RNA* (see paragraphs [0052] and [0053]; Dannenberg teaches reverse transcription of total RNA with random hexamers; total RNA would include RNA comprising intronic RNA),

*the expression of which correlates with the expression of a corresponding exonic RNA* (To the extent that this limitation is interpreted to refer to the expression of intronic RNA correlating with expression of exonic RNA (see rejection of this claim under 35 U.S.C. 112(2) above), this limitation is inherent because the steady-state level of any intron would correlate with the steady state level of any exon from the same gene, as the two are transcribed as a unit and thus have the same rate of synthesis. The steady state level of a sequence, whether intron or exon, would be determined by its rate of synthesis and its rate of degradation. Therefore, even if the intron sequence degraded

more rapidly than the exon sequence, the steady state levels of intron and exon would still correlate. For example, a given intron/exon pair might have a correlation of 1:5; i.e. the steady state level of the exon is 5 times greater than the steady state level of the intron. Further evidence that levels of intron correlate with levels of exon are provided by the teachings of Duvick, discussed below.),

*with at least one set of PCR primers and probe corresponding to said RNA* (see paragraph [0054])*; and*

*(b) performing PCR amplification* (see paragraph [0054]).

With regard to claim 56, Dannenberg teaches PCR primers and probes designed based on unique sequences in the target genes of interest (see paragraphs [0021] and [0055]).

With regard to claim 57, Dannenberg teaches extracting total RNA (which would *represent multiple genes of interest*) from formalin-fixed, paraffin-embedded tissue (see paragraphs [0021], [0051] and [0053]). Such RNA tends to be highly fragmented (see paragraph [0003] of instant specification). Therefore, this limitation is considered inherent to the disclosure of Dannenberg.

With regard to claim 58, Dannenberg teaches contacting the sample with a pool of PCR primers and probes (see paragraphs [0054] and [0055]).

With regard to claim 61, Dannenberg teaches tissue samples which are tumor tissue biopsies (see paragraph [0049]).

With regard to claim 62, one of ordinary skill in the art would have inferred that the tissue samples were obtained from human patients based on Dannenberg's

corresponding use of human cell lines (which themselves represent samples derived from biopsies of human patients; see paragraph [0048]).

With regard to claim 63, Dannenberg teaches colon cancer (see Example 6, paragraphs [0088]-[0089] and figure 8).

With regard to claim 64, Dannenberg determines the expression levels of the RNA transcripts of the genes of interest (see Example 5, paragraph [0087]).

With regard to claim 65, Dannenberg teaches correlation of differential expression of the TS gene to response to 5-FU/Leucovorin (see Example 5, paragraph [0087]).

With regard to claim 66, Dannenberg measures expression of TS, TP, COX2 and VEGF, all of which are listed in figure 6 of the instant application (see Example 6, paragraphs [0088]-[0089] and figure 8).

With regard to claim 102, Dannenberg teaches reverse transcription of total RNA extracted from formalin-fixed, paraffin-embedded tissue with random hexamers (see paragraph [0053]). Thus Dannenberg's method "uses" both intronic and exonic polynucleotide sequences (since random hexamers would comprise all possible 6-mers, including those found in intronic as well as exonic sequences).

Dannenberg does not teach:

Claim 25: *detecting a polynucleotide-intronic RNA complex* (note however that Dannenberg does teach reverse transcription of total RNA with random hexamers, which would meet claim 25 limitations of providing a polynucleotide complementary to

intronic RNA and hybridizing the polynucleotide to intronic RNA to form a polynucleotide-intronic RNA complex).

Claim 36: *quantifying the expression of intronic RNA.*

Claim 55: *contacting the sample with a set of PCR primers and probe corresponding to intronic RNA.*

Claims 56 and 58: *wherein said PCR primers and probe are designed based upon intronic RNA.*

Duvick teaches detecting intron RNA and that the level of detected intron RNA would be proportional to (i.e. correlate with) the transcription rate (see column 5, last paragraph, continuing in column 6).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention of the instant application was made to incorporate the suggestion of Duvick (to detect gene expression by detecting intron sequences) into the general method taught by Dannenberg for using PCR probes and primers to amplify RNA extracted from formalin-fixed paraffin-embedded tissue. By modifying the method of Dannenber to use PCR primers and probes based on intron sequences, one would have arrived at the claimed invention. Duvick teaches that "[r]ecent data from mammalian cells indicates that intron RNAs, after splicing, persist in the cell with reasonable half-lives, contrary to what was previously thought" (column 5 line 67 through column 6 line 3). Therefore, the idea of measuring gene expression by detecting intron RNA was clearly known in the art. One would have been motivated to choose intron RNA when practicing the general method taught by Dannenberg,

because in light of Duvick's disclosure, intron RNA sequences simply represent equivalents of the exon RNA sequences for the purpose of detecting an RNA transcript.

Claims 67, 68 and 71-74 are rejected under 35 U.S.C. 103(a) as being unpatentable over Dannenberg et al (US 2001/0029018 A1) in view of Duvick (USPN 7,026,123 filed August 28, 2002, priority date August 29, 2001) as applied to claims 25, 28-30, 32, 33, 36-39, 44, 55-58, 61-66 and 102 above, and further in view of Dai et al (US 2003/0224374 A1, filed June 14, 2002, priority date June 18, 2001).

The teachings of Dannenberg and Duvick have been discussed. Dannenberg and Duvick do not teach applying the method of claim 63 to a sample of breast cancer (claims 67-68 and 71-74) or invasive breast cancer (claims 67, 68, 73 and 74). Dannenberg and Duvick do not teach determining whether the likelihood of long-term survival of said patient without recurrence of breast cancer has increased or decreased (claims 67-68 and 71-74). Dannenberg and Duvick do not teach normalizing expression levels of genes of interest against expression levels of all RNA transcripts (claims 68, 72 and 74).

With regard to claims 67, 71 and 73, Dai teaches measuring gene expression of CEGP1 (the gene corresponding to elected SEQ ID NO:1)(see paragraph [0114]: "...comparing the level of expression of the markers listed in Table 5 in a sample ..."; note CEGP1 is listed in Table 5, page 45 of the published application, as NM_020974/SEQ ID NO:1844; cf Table 6, page 46 of the published application: NM_020974 is CEGP1) in a sample of invasive breast cancer (Applicant does not

define "invasive breast cancer", and Dai teaches selecting samples on the basis of "primary invasive breast carcinoma"; see paragraph [0178]). CEGP1 is "a gene or gene set" recited by the claims. Furthermore, Dai teaches statistical analysis (see paragraph [0120]: "...the expression level of each of the markers can be normalized by the average expression level of all the markers..."; this constitutes a "statistical analysis"). Additionally, Dai teaches determining whether the likelihood of long-term survival without the recurrence of breast cancer has increased or decreased (see for example paragraph [0114]: "...the invention provides for method of determining whether an individual afflicted with breast cancer will likely experience a relapse..."; i.e. likelihood of long-term survival without the recurrence of breast cancer has decreased).

With regard to claims 68, 72 and 74, Dai teaches "...the expression level of each of the markers can be normalized by the average expression level of all the markers..." (see paragraph [0120]).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention of the instant application was made to apply the method suggested by the combination of Dannenberg and Duvick (i.e. the method of analyzing gene expression in a fixed, embedded tissue sample using primers/probes to intronic RNA sequences) to measuring the expression of CEGP1 in a sample of invasive breast cancer, perform statistical analysis, and render a prognosis as taught by Dai, thus arriving at the claimed invention. One would have been motivated to apply the method suggested by Dannenberg and Duvick in this way, because Dai teaches that:

> "Accurate prognosis, or determination of distant metastasis-free survival could allow the oncologist to tailor the administration of adjuvant chemotherapy, with women having poorer prognoses being given the most aggressive treatment. Furthermore, accurate

> prediction of poor prognosis would greatly impact clinical trials for new breast cancer therapies, because potential study patients could then be stratified according to prognosis. Trials could then be limited to patients having poor prognosis, in turn making it easier to discern if an experimental therapy is efficacious." (paragraph [0010])

Claims 59 and 60 are rejected under 35 U.S.C. 103(a) as being unpatentable over Dannenberg et al (US 2001/0029018 A1) in view of Duvick (USPN 7,026,123 filed August 28, 2002, priority date August 29, 2001) as applied to claims 25, 28-30, 32, 33, 36-39, 44, 55-58, 61-66 and 102 above, and further in view of Dai et al (US 2003/0224374 A1, filed June 14, 2002, priority date June 18, 2001), GenBank GI:8052236 (May 22, 2000, [online], [retrieved on 2007-01-30], retrieved from the Internet: <URL: http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?8052236:OLD03:209064>, page numbers added by the examiner for reference), and Buck et al (1999).

The teachings of Dannenberg and Duvick have been discussed. Dannenberg and Duvick do not teach the method of claim 58 wherein said pool comprises at least SEQ ID NOS:14, 15, and 16 (the elected SEQ ID NOS from figure 2). It is noted that SEQ ID NOS:14, 15 and 16 represent primers and probes corresponding to intron sequences in the CEGP1 gene (see instant figure 2).

Dai teaches measuring gene expression of CEGP1 (the gene corresponding to elected SEQ ID NO:1)(see paragraph [0114]: "...comparing the level of expression of the markers listed in Table 5 in a sample ..."; note CEGP1 is listed in Table 5, page 45 of the published application, as NM_020974/SEQ ID NO:1844; cf Table 6, page 46 of the published application: NM_020974 is CEGP1).

GenBank GI:8052236 discloses a genomic sequence comprising CEGP1 gene, including introns. The sequences of SEQ ID NOS: 14, 15 and 16 are found within intron

1 of the CEGP1 gene disclosed by GenBank GI:8052236 (page 12). Specifically, SEQ ID NO:14 corresponds to nucleotides 19135-19152, SEQ ID NO:15 corresponds to the complement of nucleotides 19182-19205, and SEQ ID NO:16 corresponds to nucleotides 19160-19174.

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention of the instant application was made to apply the method suggested by the combination of Dannenberg and Duvick (i.e. the method of analyzing gene expression in a fixed, embedded tissue sample using primers/probes to intronic RNA sequences) to measuring the expression of CEGP1 in a sample of invasive breast cancer. One would have been motivated to do this because Dai teaches that CEGP1 is a preferred marker gene whose expression correlates with prognosis (see paragraph [0114] and Tables 5 and 6), and because Dai teaches that:

> "Accurate prognosis, or determination of distant metastasis-free survival could allow the oncologist to tailor the administration of adjuvant chemotherapy, with women having poorer prognoses being given the most aggressive treatment. Furthermore, accurate prediction of poor prognosis would greatly impact clinical trials for new breast cancer therapies, because potential study patients could then be stratified according to prognosis. Trials could then be limited to patients having poor prognosis, in turn making it easier to discern if an experimental therapy is efficacious." (paragraph [0010])

It would also have been obvious to select primers and probes corresponding to SEQ ID NOS:14, 15 and 16 from the known sequence of the CEGP1 gene because the primers and probes of SEQ ID NOS:14, 15 and 16 simply represent equivalents to the probes and primers suggested by Dai for the purpose of detecting CEGP1 (see paragraphs [0145], [0146] and [0154], last sentence).

Buck expressly provides evidence of the equivalence of primers. Specifically, Buck invited primer submissions from a number of labs (39) (page 532, column 3), with

69 different primers being submitted (see page 530, column 1). Buck also tested 95 primers spaced at 3 nucleotide intervals along the entire sequence at issue, thereby testing more than 1/3 of all possible 18 mer primers on the 300 base pair sequence (see page 530, column 1). When Buck tested each of the primers selected by the methods of the different labs, Buck found that EVERY SINGLE PRIMER worked (see page 533, column 1). Only one primer ever failed, No. 8, and that primer functioned when repeated. Further, EVERY SINGLE CONTROL PRIMER functioned as well (see page 533, column 1). Buck expressly states "The results of the empirical sequencing analysis were surprising in that nearly all of the primers yielded data of extremely high quality (page 535, column 2)." Therefore, Buck provides direct evidence that all primers would be expected to function, and in particular, all primers selected according to the ordinary criteria, however different, used by 39 different laboratories. It is particularly striking that all 95 control primers functioned, which represent 1/3 of all possible primers in the target region. This clearly shows that every primer would have a reasonable expectation of success.

Regarding the obviousness of substituting equivalents known for the same purpose, MPEP 2144.06 states: "In order to rely on equivalence as a rationale supporting an obviousness rejection, the equivalency must be recognized in the prior art, and cannot be based on applicant's disclosure or the mere fact that the components at issue are functional or mechanical equivalents." In this case, Buck clearly establishes that different primers derived from a known sequence represent equivalents in terms of functioning as a primer for that sequence.

***Conclusion***

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Samuel Woolwine whose telephone number is (571) 272-1144. The examiner can normally be reached on Mon-Fri 9:00am-5:00pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on (571) 272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

scw

JEFFREY FREDMAN
PRIMARY EXAMINER
2/1/07